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NATURAL RESOURCES DEFENSE COUNCIL

May 21, 2003

NIEHS
MD B2-01
P.O. BOX 12233
RTP, NC 27709-2233

Dear Drs. Ken Olden and Chris Portier;

We write to express our concern that the p53 deficient (p53+/- heterozygous) transgenic mouse model is unreliable at detecting potential nongenotoxic carcinogens, and yet is being mis-used to declare potential nongenotoxic carcinogens as non carcinogenic by the NTP. We represent The Natural Resources Defense Council (NRDC). NRDC uses law, science, and the support of more than 500,000 members nationwide to protect the health of the American public.

Acesulfame potassium (also called acesulfame K) is a non-calorie artificial sweetener sold under the brand name Sunett™ by Nutrinova, Inc. A review of the sparse carcinogenicity data indicated that an apparently adequate 18-month mouse carcinogenicity study had been conducted with acesulfame potassium.[i] Because acesulfame potassium is not biotransformed after absorption and was negative in both a mouse and rat carcinogenicity study, it was considered a candidate for a negative control in the evaluation of Tg.AC[ii] and p53 mouse models. However, this is a poor choice of a negative control given that there has not been an adequate, validated full two-year cancer bioassay performed for acesulfame K. Both the rat (Sinkeldam et al, 1991) and mouse (Beems et al, 1991) study results are available only from an industry report titled, *Acesulfame-K*, and are not available through the published scientific literature.[iii]

We are concerned that negative results in a transgenic model that is unable or unreliably able to detect non-genotoxic chemicals such as acesulfame K, are being mis-interpreted as evidence of lack of carcinogenicity. There are several known carcinogens that test negative in the p53 mouse model, including 17B-estradiol, TCDD (dioxin), glycidol, chloroprene, phenacetin, oxymetholone, pentachlorophenol

In addition, aspartame was one of the test agents selected for the evaluation of the genetically manipulated mouse strains, Tg.AC, and p53, with similarly negative results.[iv] However, aspartame has been evaluated in conventional rodent cancer studies (non NTP studies) and, it has been argued in the published literature that the studies showed an elevated risk of brain tumors. Thus, we are concerned that the predictably negative results of these trangenic mouse studies are being interpreted as evidence of non carcinogenicity, rather than more appropriately as evidence of the inability of the model to reliably detect nongenotoxic carcinogens. For example, has the NTP demonstrated that a 9-month study in the p53 transgenic mouse would detect brain carcinogens (i.e., positive controls)? The NTP should recognize that a study of an agent in an inapproriate model (e.g., nongenotoxic carcinogens in the p53 model) is not a reliable test of that agent's carcinogenic potential. Hence, the title of this technical report is misleading and the application of NTP's levels of evidence, as used for conventional studies in rats and mice, is a misrepresentation and misinterpreation of the data.

Attempts to validate a model through negative findings, and then declaring the agents as non-carcinogens is a circular argument that will give undue credibility to studies in models that have not been adequately validated for their ability to detect environmental and occupational

carcinogens. In fact, industry recently pushed the NTP to withdraw the positive calls on acrylates in the Tg.AC mouse studies, with claims that the transgenic models are not robust enough to classify agents with confidence. And, an industry committee that evaluated alternative cancer models for ILSI concluded that the available data do not support the use of transgenic models in place of the two-year standard bioassay.[v] Concerning the p53+/- model the committee concluded that this model "could identify some genotoxic carcinogens."

We suggest that based on the definitions for two-year bioassay levels of evidence, the findings of the aspartame and acesulfame K studies should be labeled as an "inadequate study of carcinogenic activity," based on the NTP definition for an inadequate study: "major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity." This is certainly scientifically credible. Reading any more into these results would stretch the scientific data indefensibly and mislead the public into believing that the NTP had adequately evaluated the carcinogenic potential of aspartame and acesulfame K in validated models.

We ask you to share this letter with members of the NTP Technical Reports Peer-Review Panel.

Respectfully,

Jennifer Sass, Ph.D.
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Sent by email to olden@niehs.nih.gov

Sent by email to portier@niehs.nih.gov

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[i] Toxicity Studies Of Acesulfame Potassium (CAS NO. 55589-62-3) in FVB/N-TgN(v-Ha-ras)Led (Tg.AC) Hemizygous Mice and Carcinogenicity Studies of Acesulfame Potassium in B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient Mice (Feed Studies). http://ntp-server.niehs.nih.gov/htdocs/GMM-studies/GMM02.html

[ii] The Tg.AC transgenic line was produced in FVB/N mice by pronuclear injection of a v-Ha-ras transgene linked to a fetal zeta-globin promoter and an SV40 polyadenylation/splice sequence.

[iii] See p. 47 of NTP technical report on Acesulfame Potassium, available electronically at http://ntp-server.niehs.nih.gov/htdocs/GMM-studies/GMM02.html

[iv] 39-Week (Dosed-Feed) (C99033), Subch ToxReview. Mice:P53 +/- (C57BL/6) (Transgenic); MICE:TGAC (FVB/N). Hemizygous (Transgenic). Dose: 0, 3125, 6250, 12500, 25000 OR 50000 ppm in Feed; 15 animals/dose/sex

[v] Takaoka M, Sehata S, Maejima T, Imai T, Torii M, Satoh H, Toyosawa K, Tanakamaru ZY, Adachi T, Hisada S, Ueda M, Ogasawara H, Matsumoto M, Kobayashi K, Mutai M, Usui T.
Interlaboratory comparison of short-term carcinogenicity studies using CB6F1-rasH2 transgenic mice. Toxicol Pathol. 2003 Mar-Apr;31(2):191-9.